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EVIDENCE SUPPORTING A ROLE FOR ENDOGENOUS VASOPRESSIN IN NATURAL SUPPRESSION OF FEVER IN THE SHEEP

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SUMMARY

1. The antipyretic effect of arginine vasopressin (AVP) introduced into the brain by push–pull perfusion was investigated in the sheep.

2. Control perfusions with sucrose solutions had no effect on fevers induced by a bacterial endotoxin. Sucrose solutions containing AVP (4·0 μg/ml.) perfused at 40 μl./min had significant antipyretic activity, reducing the two peaks of the fever but had no effect on resting body temperature.

3. Loci in which AVP induced antipyresis were limited to the septal region about 2–3 mm anterior to the anterior commissure.

4. The amounts of AVP in perfusates from the septal region correlated negatively with changes in body temperature.

5. AVP administered I.V. did not lower fever.

6. AVP plasma levels correlated negatively with fever magnitude following premature birth induced by dexamethasone.

INTRODUCTION

Fever in response both to bacterial endotoxin, and to endogenous pyrogen, is reduced in the ewe from about 4 days before, until at least 5 hr after parturition (Kasting, Veale & Cooper, 1978*a*, *b*). During this time, the ewe remains nearly afebrile in response to relatively large I.V. doses of both pyrogens. Similarly, the newborn lamb fails to respond to these pyrogens with a fever. The ability to respond with a fever to pyrogens, after parturition, develops within a similar time period of about 32 hr in both the ewe and lamb. The ability of the ewe to thermoregulate during this period of reduced febrile response seems unimpaired. The inability of ewe and lamb to get fevers suggests a similar mechanism for the diminished response. There is evidence that germ-free animals fail to respond to bacterial endotoxin with fever for several weeks after birth (Podoprigora, 1978), and this supports the view that, in the lamb, one component of the development of the fever process is 'sensitization' (Pittman, Cooper, Veale & Van Petten, 1973, 1974). In addition, the deficit in the sequence of events which usually leads to fever appears to occur after the synthesis and release of endogenous pyrogen. One hypothesis which might account

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for the lack of fever in the near-term ewe and new-born lamb is that there is increased synthesis of some endogenous substance that can suppress fever to both types of pyrogen without having an effect on normal thermoregulation. If this were so, an increased amount of this substance should occur in both ewe and lamb at about the time of birth.

The hormonal environment of the pregnant and near-term sheep, as well as its fetus has been well studied since the development of a preparation enabling fetal arterial and venous cannulation *in utero*. The timing of known hormone fluctuations has been compared with the time course of the decreased level of induced fevers. The reported arginine vasopressin (AVP) levels in the plasma (Alexander, Bashore, Britton & Forsling, 1974) appeared to be most consistent with a possible role in the suppression of fever described above. AVP levels in the plasma rise about 4 days before parturition in both the ewe and fetus, reaching higher levels in the fetus. It has been demonstrated that AVP does not cross the placenta to any extent (Alexander *et al.* 1974; Skowsky, Bashore, Smith & Fisher, 1973). It is likely, therefore, that AVP is secreted independently in the brains of both ewe and fetus in greater quantities during this period.

There are several studies showing a relation between AVP and thermoregulation. The body temperatures of rats given a large dose of AVP, both intraperitoneally (I.P.) and intravenously (I.V.), fell (Okuno, Yamamoto & Itoh, 1965). The mechanism of this hypothermic effect was not elucidated, however, and the authors concluded that the hormone was acting peripherally. Increases in body temperature, due to exposure to high ambient temperatures, were associated with increases in plasma AVP (Forsling, Ingram & Stanier, 1976). When the basal forebrain area of dogs was heated to 1·5 °C above normal, AVP levels in plasma increased (Szczepanska-Sadowska, 1974); the most significant increase occurred when the preoptic region and the ventro-lateral septum were heated.

Clinically, several disorders may be accompanied by inappropriate secretion of AVP but an association of these conditions with fever or thermoregulatory abnormalities has not been documented.

Exogenous pyrogens (Bennett, Petersdorf & Keene, 1957; Sheth & Borison, 1960; Villablanca & Myers, 1965) and endogenous pyrogens (Cooper, Cranston & Honour, 1967; Jackson, 1967) have been shown to act principally in the preoptic/anterior hypothalamic region. Antipyretics have also been shown to act in the preoptic area (Cranston & Rawlins, 1972; Chowers, Conforti & Feldman, 1968; Vaughn, Veale & Cooper, 1979). This is the same region which, when heated, leads to AVP release (Szczepanska-Sadowska, 1974). It was therefore of interest to see if exposure of this or adjacent parts of the brain to AVP had any effect on the ability of a sheep to become febrile in response to bacterial pyrogen.

These experiments also sought to examine whether the amount of AVP (if any) released into the extracellular spaces of the brain in the regions which respond to AVP with an antipyresis, could be correlated with body temperature changes.

In a third series of experiments, labour was induced artificially to determine if sheep would have a reduced febrile response similar to that accompanying normal delivery and whether AVP levels in the plasma might parallel those changes.

METHODS

Series 1

Nine Suffolk, Dorset and cross-bred mature female sheep were used for perfusion experiments. They were shorn and allowed to acclimate to laboratory conditions at an ambient temperature of 19·0 ± 1·5 °C. Sheep were implanted stereotaxically with 17 gauge stainless-steel cannulae directed towards several regions of the hypothalamus and septum, and the cannulae were fixed to the skull with dental cement. A polyethylene cap was attached to the skull by steel screws around the cannulae to protect them and to keep the preparation clean. Indwelling stylets were inserted to occlude the cannulae when they were not in use and to keep them clean. Sheep were given 5 ml. penicillin (Derapen C, Ayerst Laboratories) post-operatively and at least 7 days elapsed before the experiments began. Sheep stood throughout the experimental period, loosely held with a neck restrainer to which they had become accustomed, to limit movement of their heads.

The body temperature of the animals was measured by thermistor probes (Yellow Springs Inst.) inserted at least 10 cm into the vagina to obtain a more stable reading than rectal temperature. The body temperature was recorded continuously on a pen recorder (Beckman Inst. – Type RM Dynograph Recorder) for at least 30 min before injection of endotoxin and for 200 min thereafter. The normal resting body temperatures of the sheep were in the range of 39·0–39·9 °C at the start of all experiments. Other physiological changes, including respiratory rate, posture and shivering, were assessed by observation.

Injection of bacterial pyrogens and the start of push-pull perfusion occurred simultaneously. Perfusions with different substances were carried out in a random order at weekly intervals. All equipment used for injection and perfusion was kept sterile and pyrogen free by baking at 180 °C for 3 hr or leaving overnight in benzalkonium chloride (Zephiran Chloride, Winthrop Laboratories). Polyethylene tubing was flushed repeatedly with sterile saline before being filled with experimental solution. All solutions were passed through a 0·22 μm filter (Millipore) before use.

A push–pull assembly was used for perfusion of brain tissue. The push–pull device consists of an outer 20-gauge stainless-steel cannula for withdrawal and an inner 27-gauge steel cannula, extending 1 mm beyond the tip of the outer cannula, for infusion. With this array, the tissue is continuously washed with the experimental solution; the volume of tissue perfused is about 1·0–1·5 mm in diameter (Myers, 1970). Matched glass syringes were mounted on a multi-channel infusion withdrawal pump (Harvard Apparatus Co.) and a constant flow of 40 μl./min was maintained for 200 min.

The solutions used for push-pull perfusion were sucrose (260 mM) and sucrose (260 mM) with AVP (0·8, 2·0, 4·0 μg/ml.). AVP was kept in a stock solution of 0·25% acetic acid 1 mg/ml. at 4·0 °C. AVP had an activity of 360 rat pressor units/mg and 360 rat antidiuretic units/mg. It was diluted out in sucrose solution immediately before each experiment. The bacterial pyrogen used was derived from *Salmonella abortus equi* (SAEP) (Difco Laboratories). A standard dose of 30 μg SAEP in 3 ml. physiological saline was injected I.V. This produced a biphasic fever with a maximum rise at 200 min of 1·36 ± 0·06 °C in unoperated adult ewes. Fever index represents the area under the fever curve calculated as °C.hr.

Perfusates were collected over half hour periods from three experiments during a fever induced by I.V. SAEP in different animals using AVP-free vehicles of sucrose (245 mM), sucrose (260 mM), and NaCl (154 mM). These perfusates were assayed for AVP. The AVP thus measured in the perfusates reflected that AVP released by brain tissue. These experiments were done in three different sheep in the brain loci which responded to AVP perfusions by a reduction of fever as determined in the first part of series 1.

Perfusion loci were identified by injection of 1 μl. 0·5% bromophenol blue into the appropriate sites in the conscious animal. The animal was given an overdose of pentobarbitone sodium and its brain perfused via the common carotid arteries with saline and 10% formal saline respectively. The brain was sectioned on a freezing microtome and the stained sites recorded.

Series 2

Twenty-three sheep received 30 μg SAEP I.V. followed by AVP infusions through jugular cannulae. They received 0·24 μg AVP/200 min (n = 5), 2·4 μg AVP/200 min (n = 13), or 24 μg AVP/200 min (n = 5) in 10 ml. physiological saline. This range of doses covered that estimated

to have entered the systemic circulation of the sheep during the brain perfusion study in series 1. Body temperature was recorded in all animals as in series 1.

Series 3

Saphenous vein cannulae were surgically implanted in the fetuses of five Suffolk sheep between 112 and 116 days gestation (Willes, Van Petten & Truelove, 1970). The cannulae were exteriorized to a pouch on the ewe's side and kept sterile. Premature delivery was induced by infusing dexamethasone (Decadron, Merck Sharp and Dohme) (1 mg/24 hr) into the fetal saphenous vein between 121 and 130 days gestation (Liggins, 1969). A sample of maternal jugular venous blood was withdrawn into heparinized syringes between 1 and 3 hr after the induced birth. The blood was centrifuged at 1500 *g* for 20 min at 4 °C and plasma frozen at −40 °C until assay.

The ewe was given the standard challenge of 30 μg SAEP I.V. immediately following the blood sample and the body temperature recorded as in series 1. The body temperatures of these sheep, at the start of the experiment, were between 39·0 and 39·9 °C.

Assay

AVP was extracted from push-pull perfusates and plasma samples by the bentonite extraction procedure according to Skowsky, Rosenbloom & Fisher (1974) and assayed with a high specificity radioimmunoassay sensitive to < 1·0 pg/ml. sample and with low cross reactivity (Moore, Lutterodt, Burford & Lederis, 1977). Synthetic AVP was kindly provided by Dr G. Moore.

RESULTS

Series 1

Four of fourteen sites tested were sensitive to AVP in that there was a decrease in the febrile response. These sites were located in the septal area of four different sheep (Fig. 3). Fig. 1 shows the fever index (°C.hr for 3 hr) and the maximum height of the fever for the four animals when perfused in sensitive sites with sucrose-only and sucrose with AVP (4·0 μg/ml.) and also compares the response of normal unoperated animals. Both the fever index and the maximum fever height were significantly lower during AVP perfusion than with either sucrose-only perfusion or no perfusion. The maximum fever height decreased from 1·13 °C with sucrose-only perfusions to 0·38 °C with sucrose and AVP perfusion. The fever index decreased from 1·93 °C.hr with sucrose-only perfusions to 0·2 °C.hr with sucrose and AVP. Unoperated controls had a maximum fever height of 1·36 °C and a fever index of 1·87 °C.hr. The changes in the sucrose and AVP perfused group were significantly different ($P < 0{\cdot}0005$) from the other two groups.

Perfusion with AVP in the sensitive sites of two animals but without SAEP challenge caused no appreciable change in normal body temperature. Previous observations have shown that there is an exchange of about 10% of solution in the push–pull perfusate with the tissue (Veale, 1971) so a probable estimate of the AVP actually entering the tissue would be 3·2 μg per side or a total of 6·4 μg over the 200 min perfusion period. This would be equivalent to 32 ng AVP/min.

Those animals receiving the AVP and not responding with a fever did not show physiological changes normally associated with a fever, i.e. shivering, huddled stance, slower respiration.

Fig. 2 illustrates a dose–response relationship in one animal to varying concentrations of AVP. The fever index (°C.hr) was calculated for 3 hr for the response of the ewe to SAEP challenge while having the septal area perfused with sucrose only, sucrose with 0·8 μg AVP/ml., sucrose with 2·0 μg AVP/ml., and sucrose with

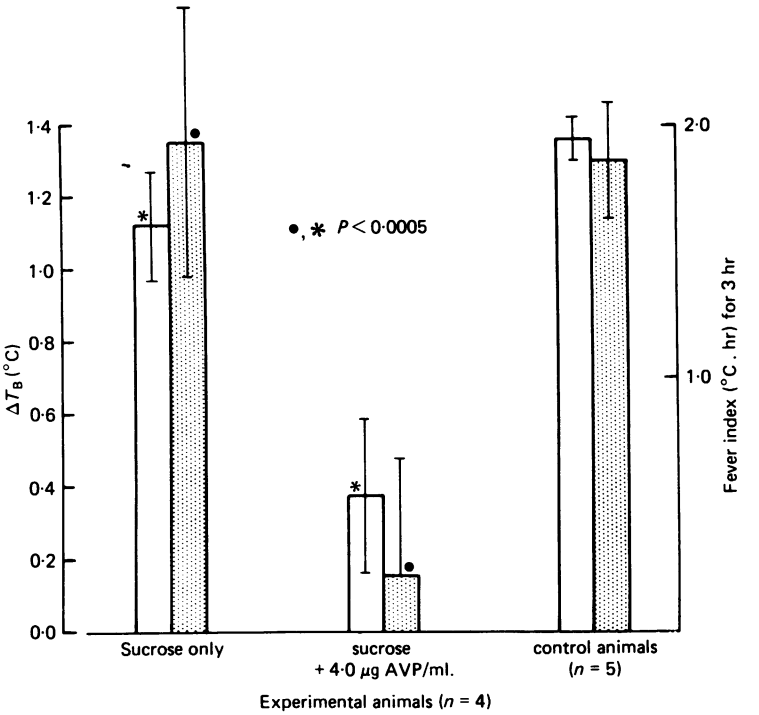

Fig. 1. Vertical bars represent the maximum fever height (open bars) during 200 min and the 3 hr fever index (°C.hr) (stippled bars) in experimental animals following push–pull perfusion (40 μl./min) with sucrose only in the septal area, and with sucrose plus 4·0 μg AVP/ml. in the same sites. Fever height and fever index were both significantly decreased when AVP was added to sucrose. The bars on the right represent the fever height and fever index in control (unoperated) animals. Vertical lines respresent mean ±S.E. of mean.

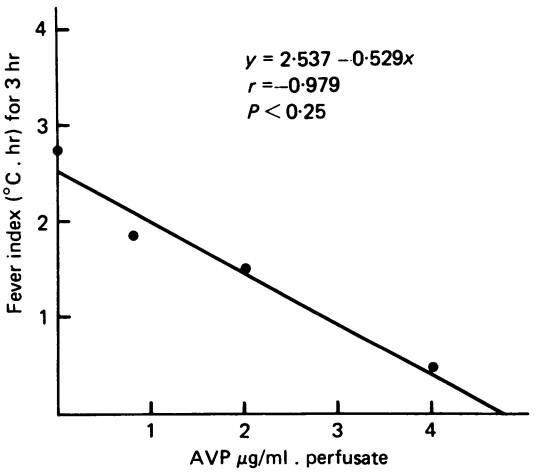

Fig. 2. Linear regression analysis of the dose–response relation between AVP in the perfusion solution and 3 hr fever index (°C.hr) for one ewe. Doses are 0, 0·8, 2·0, and 0·8, 2·0, and 4·0 μg AVP/ml. sucrose.

4·0 μg AVP/ml. This graph shows a reciprocal relationship between the area under the fever curve and the concentration of AVP in the perfusing solution.

Sucrose (260 mM) was chosen as a vehicle for the AVP because it had the least effect on normal body temperature when perfused through this area as compared to NaCl (154 mM), artifical extracellular fluid or sucrose (245 mM). The osmolality of the 260 mM-sucrose was 282 m-osmole as measured by freezing point depression.

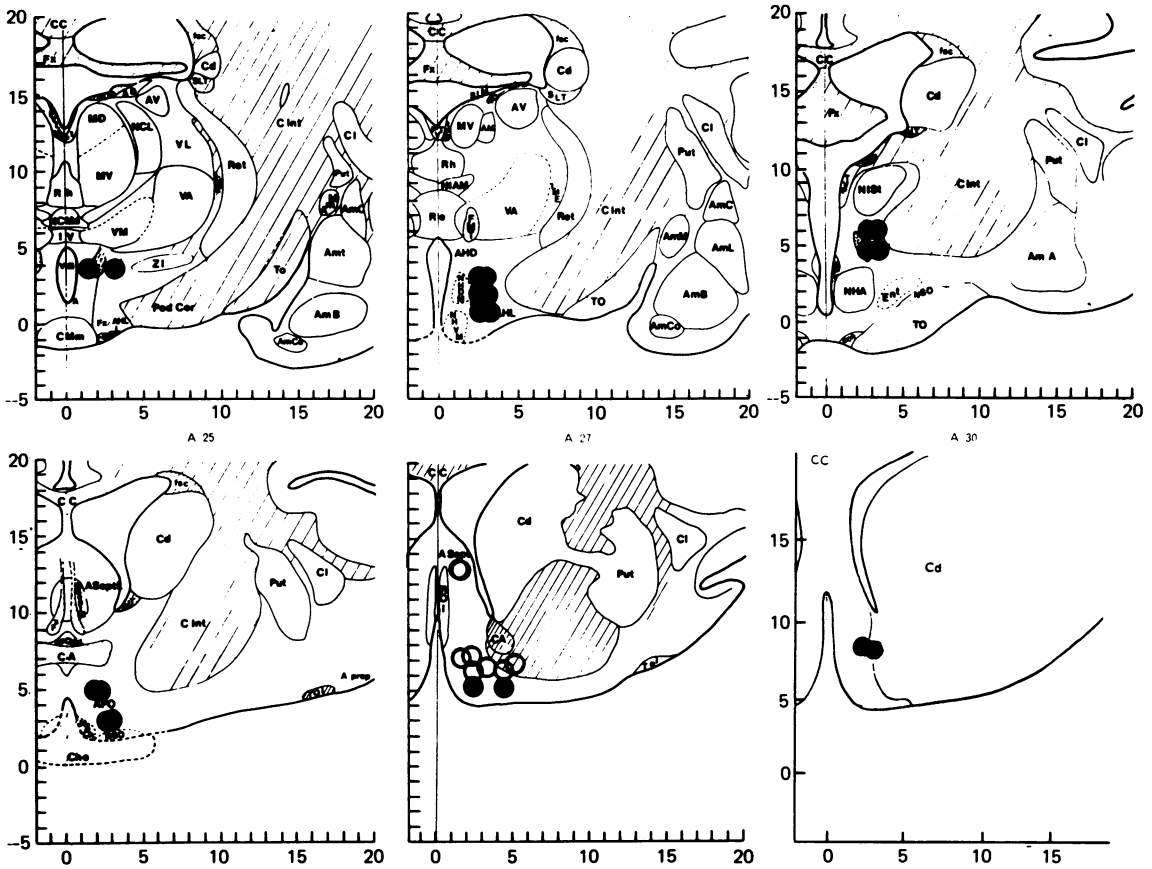

Fig. 3. Brain sections showing loci tested with AVP perfusion. Filled circles represent loci where no antipyresis was elicited and open circles represent loci where antipyresis was elicited. Loci from both sites are represented on the one side.

The locations of the sites sensitive to the AVP are shown in Fig. 3. Perfusion sites from both sides are represented on one side in the illustrations. The anatomical sections drawn are taken from Richards (1967). The filled circles represent sites not sensitive to AVP and the approximate area of tissue perfused by this technique. The sensitive sites are shown as open circles. The sensitive sites appear to be limited mainly to the septal area approximately 2–3 mm anterior to the anterior commissure. At least one of each pair of sensitive sites was in the septal area.

The perfusates from push–pull perfusions in septal areas of three sheep with various AVP-free vehicles were assayed for AVP at half hour intervals during and

after the induction of a fever. Fig. 4 shows the regression curves (least-squares method) for these experiments. The curves compare the amount of AVP assayed by radioimmunoassay in the perfusate (therefore released by brain tissue) with the change in body temperature during the same half hour over which the perfusate was collected.

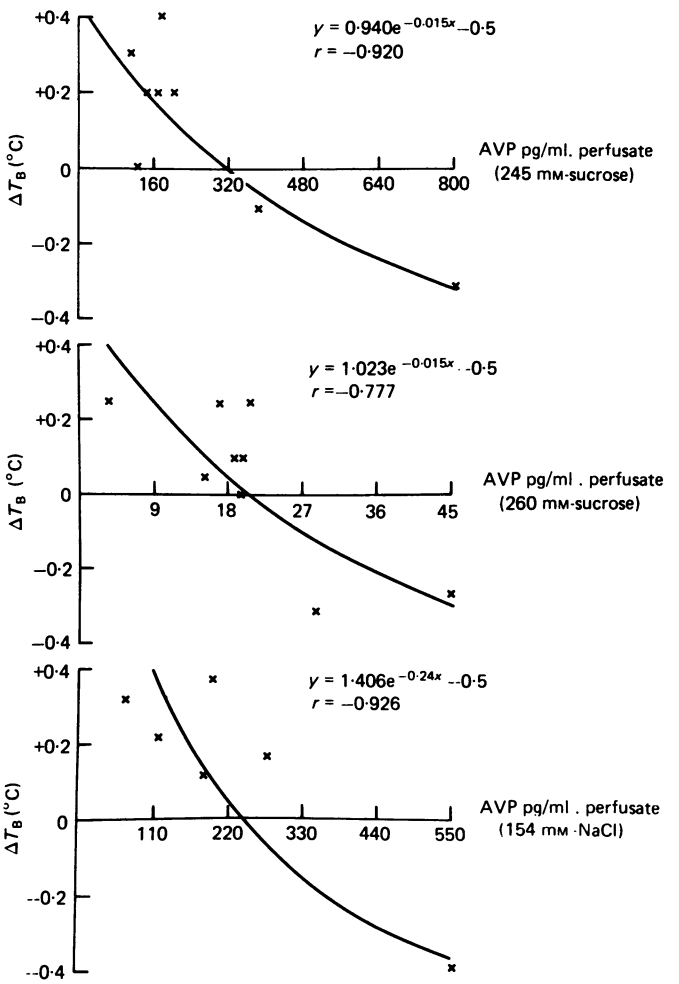

Fig. 4. Results from three experiments performed on separate sheep in which AVP in the perfusate from push–pull perfusions with three AVP-free vehicles was measured in animals that were challenged with SAEP. Each line is the best fit curve relating changes in body temperature and the concentration of AVP.

These curves show correlation coefficients of −0·920, −0·777 and −0·926. The amounts of AVP in the perfusates varied from 3·7 to 790 pg/ml. perfusate.

Fig. 5 illustrates the combination of these three experiments by expressing the AVP content of each sample as a percentage of the greatest amount of AVP released into the perfusate during that experiment. This curve has a correlation coefficient of −0·86. Thus, high AVP levels present in the extracellular fluid in this region of the

brain were associated with falls in body temperature while lower levels were associated with rises in body temperature.

Series 2

AVP at 0·24, 2·4 and 24 μg/200 min was infused I.V. in twenty-three sheep to determine if AVP was exerting its antipyretic effect by peripheral actions. The mean fevers occurring were not significantly different from each other (1·20 ± 0·13, 1·05 ± 0·17, 1·28 ± 0·20 °C, respectively) or from controls (1·36 ± 0·06 °C).

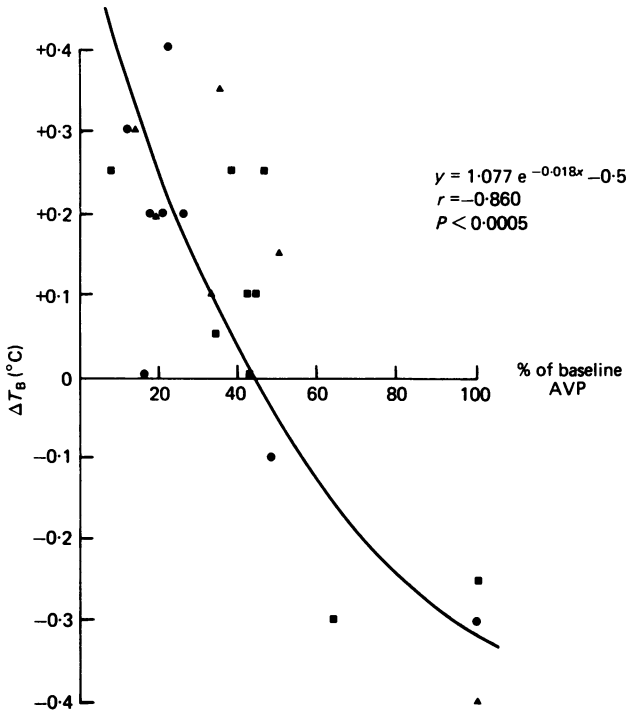

Fig. 5. This graph combines the data from Fig. 3 by making each point a percentage of the greatest AVP concentration found in each experiment. The line is the best fit curve relating the changes in body temperature and the percentages of AVP concentrations in the perfusates.

Series 3

In the third series of experiments, birth occurred at 48–92 hr after starting the infusion of dexamethasone into the fetus. Fig. 6 shows the regression line (least-squares method) comparing radioimmunoassayable AVP levels in the plasma following birth and the maximum height of the fever produced in response to SAEP. The correlation coefficient −0·91 is significant ($P < 0{\cdot}025$) even though numbers are few ($n = 5$). Mean AVP levels in the pregnant sheep before infusion of dexamethasone were 3·13 ± 0·35 pg/ml. plasma. This agrees well with the regression curve in Fig. 6 with regards to the expected height of the fever (1·36 °C) in these

animals. The values of AVP in plasma after birth were 3·02, 4·60, 9·06, 12·08 and 14·38 pg/ml. The fever heights were 1·3, 1·3, 0·65, 0·85 and 0·2 °C, respectively. None of the fetuses lived long enough to be challenged with the bacterial pyrogen.

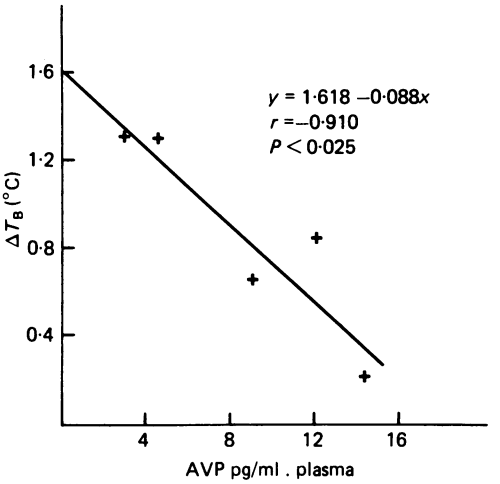

Fig. 6. The relation between the plasma levels of AVP in the ewe and the subsequent maximum fever height (°C), in the ewe, in response to 30 μg SAEP I.V. immediately following dexamethasone induced birth in the sheep. A linear regression analysis was performed on the data.

DISCUSSION

The object of the first series of experiments was to determine if AVP has antipyretic activity when perfused in the brain of the sheep. The results indicate that AVP perfused in the septal region in amounts estimated to be about 6·4 μg over a period of 200 min can act to reduce fever significantly. This compares, on a weight basis, to the antipyretic effects of salicylate at 6–30 μg (Cranston *et al.* 1972) or of cortisol at 2·5 μg (Chowers *et al.* 1968) administered centrally in the rabbit.

The antipyresis did not appear to be accomplished by a lowering of normal body temperature. This is consistent with the fact that the pre- and post-partum ewe and new-born lamb both regulate at around normal body temperatures during the period of antipyresis (unpublished observations). The ewe and new-born have not been tested for febrile responses at different ambient temperatures. However, the fact that lambs can survive when born at ambient temperature of −40 °C indicates an efficient ability to thermoregulate (Alexander, 1975; Pittman *et al.* 1974).

The detection of AVP in perfusates during fever and the negative correlation of its concentration with changes in body temperature is an interesting association but, by itself, suggests neither cause nor effect. However, AVP was released in the septal tissue in larger quantities in association with falling body temperature. The large variations in absolute amounts of AVP in the perfusates in the three experiments could be due to changes in either osmolality of the solutions or to variations in the proximity of cannulae tips to AVP releasing neurones. The latter seems more likely

since the decrease in osmolality from perfusion of 260 mM-sucrose to perfusion of 245 mM-sucrose resulted in more AVP release, and this is opposite to what might be expected if AVP release was dependent on the osmolality of the surrounding fluid.

The detected AVP levels in the plasma were negatively correlated with the subsequent height of the fever in ewes immediately following induced birth. This suggests that, at least in these particular experiments, AVP levels in the blood may have reflected central activity of AVP with regards to its involvement in fever. It would be suspected, however, that AVP levels in the blood would normally reflect neurohypophyseal activity as well. However, even with this relation between AVP blood levels and fever magnitude, the I.V. infusion experiments showed that AVP was not acting peripherally.

Sucrose solutions caused no antipyresis, whereas, low levels of AVP applied to the septal region had a profound antipyretic effect in the sheep. To implicate the septum in fever, it would seem necessary to postulate pathways linking the septum and the pre-optic regions. Reciprocal pathways connecting the pre-optic region of the hypothalamus and septal regions are a prominent component of the medial forebrain bundle (Nauta & Haymaker, 1969), thus satisfying this requirement. In addition, thermosensitive neurones have been found in the septal area (Cunningham, Stolwijk, Murakami & Hardy, 1967; Nakayama & Hardy, 1969).

A study which evaluated the effects of salicylate on septal and preoptic neurones found that equal numbers of septal and preoptic neurones were responsive to salicylate applied ionophoretically. Neurones displayed increased firing rate (28·8%), decreased firing rate (9·6%) or were unaffected (61·5%) (Beckman & Rozkowska-Ruttimann, 1974).

Thermosensitive septal and preoptic neurones react to I.V. pyrogen by an increase in their thermosensitivity (Eisenman, 1969). Electrical stimulation studies in cats (Stuart, Kauramura & Hemingway, 1961) determined that shivering could be produced by ventro-lateral septal stimulation and inhibited by ventro-medial septal stimulation even though the required stimulus was greater than in certain other hypothalamic areas. Another study (Andersson, 1957) found that septal electrical stimulation in goats elicited cold defence reactions.

The route by which AVP would reach this area *in vivo* may be by direct release from terminals in this region. Pathways from the supraoptic region or from other vasopressin synthesizing neurones to this area would be necessary. Septal peptidergic neurones have been described in many vertebrates, including mammals (Sterba, 1974). A recent immunocytochemical study using AVP antisera describes putative AVP containing punctate pericellular fibres in the lateral septum of the rat (Buiys, Swaab, Dogterom & van Leeuwen, 1978). Another study has shown suprachiasmatic-septal pathways containing AVP (Sofroniew & Weindl, 1978).

A second possibility is that AVP could reach the septum via the c.s.f. or that there are high local blood levels of AVP. Finally, it is important to note that the perfusate collected from control surcrose-only perfusions in these sensitive sites contained detectable AVP, thus suggesting these areas normally contain or release AVP.

Alternatively, AVP may be diffusing to some site more accessible from the septum than from the other sites tested. For example, the organum vasculosm laminae terminalis is only 2–3 mm distant and contains AVP (Zimmerman & Antunes, 1976).

It could also be that AVP may be interacting with receptors for some other molecule and it is similar enough to elicit a response.

The AVP was not acting peripherally. Evidence supporting this statement is that perfusions of other brain areas did not result in antipyresis even though the likelihood of the AVP passing into the circulation should be equally as great. Secondly, I.V. infusion of 0·24, 2·4 or 24·0 μg AVP/200 min produced no significant antipyresis. The fevers appeared slightly lower but were not statistically different from controls. Thus, we infer that AVP was acting centrally to produce its antipyretic effect.

There is evidence that antipyretic drugs, including acetaminophen (Nusynowitz & Forsham, 1966), acetylsalicylic acid and indomethacin (Silverstein, Feldman, Henderson & Engelman, 1975) have antidiuretic actions on the kidney. In the case of acetylsalicylic acid and indomethacin, this may be by action on the distal tubules to increase water resorption. Furthermore, prostaglandin E_1 (PGE_1) has been shown to antagonize the effect of AVP on osmotic water movement and may act at the same site (Grantham & Orloff, 1968; Beck, Kanecko, Zor, Field & Davis, 1971; Parisi & Piccinni, 1972; Hall & Martin, 1974; Anderson, Berl, McDonald & Schrier, 1975). PGE_1 is a potent pyrogenic agent and may be involved in the genesis of fever, although the evidence of this role is not clear (Veale, Cooper & Pittman, 1977). These reports of a similar action of AVP and exogenous antipyretic drugs on kidney tubules and an antagonistic relation between AVP and PGE_1 is interesting with respect to the antipyretic effects of AVP reported here.

The original question prompting the experiments reported here was concerned with the cause of the antipyresis in near-term ewes and new-born lambs. It appears that high AVP levels in ewe's blood show a time course consistent with its involvement in this phenomenon (Alexander *et al.* 1974). We have demonstrated, furthermore, that in specific sites in the brain, AVP is an effective antipyretic in relatively low amounts. We have also demonstrated that AVP released into brain extracellular space in these same regions is negatively correlated with changes in body temperature during fever. Finally, we have demonstrated that a negative correlation also exists between blood levels of AVP and subsequent fever height in ewes following induced premature birth.

It is possible, therefore, that AVP may be involved in a form of natural antipyresis occurring around the time of parturition in the ewe and in the new-born lamb. This evidence is, however, only suggestive and does not prove that AVP is involved nor does it exclude other factors that may be involved. Further work is needed on these phenomena to establish if they are indeed related physiologically and not just experimentally.

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